J Clin Periodontol 2006; 33: 605–611 doi: 10.1111/j.1600-051X.2006.00961.x

Journal of Clinical Periodontology

Evaluation of t-PA, PAI-2, IL-1β and PGE_2 in gingival crevicular fluid of rheumatoid arthritis patients with periodontal disease

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Bıyıkoğlu B, Buduneli N, Kardeşler L, Aksu K, Öder G, Kütükçüler N. Evaluation of t-PA, PAI-2, IL-1β and PGE_2 in gingival crevicular fluid of rheumatoid arthritis patients with periodontal disease. J Clin Periodontol 2006; 33: 605–611. doi: 10.1111/j.1600-051X.2006.00961.x.

Abstract

Aims: This study was undertaken to compare periodontal conditions, gingival crevicular fluid (GCF) levels of tissue-type plasminogen activator (t-PA), its inhibitor plasminogen activator inhibitor-2 (PAI-2), interleukin-1β (IL-1β), prostaglandin E_2 (PGE_2) in rheumatoid arthritis (RA) patients and control groups.

Methods: Twenty-three RA patients, 17 systemically healthy patients with periodontal disease (PD), and 17 systemically and periodontally healthy subjects were recruited. GCF samples were obtained from two single-rooted teeth. Full-mouth clinical periodontal measurements were recorded at six sites/tooth. GCF samples were analysed using relevant ELISA kits. Data were tested statistically by appropriate tests.

Results: Total amounts of t-PA, PAI-2 and PGE_2 in GCF samples of the healthy control group were significantly lower than the other groups ($p<0.05$). The RA group exhibited a higher total amount of t-PA in GCF samples than the PD group ($p<0.05$). PAI-2, IL-1β and PGE_2 total amounts were similar in RA and PD groups ($p>0.05$).

Conclusion: The coexistence of RA and periodontitis does not seem to affect clinical periodontal findings or systemic markers of RA. Similar inflammatory mediator levels in RA and PD groups, despite the long-term usage of corticosteroids, non-steroidal anti-inflammatory drugs, suggest that RA patients may have a propensity to overproduce these inflammatory mediators.

Key words: ELISA; gingival crevicular fluid; IL-1β; PAI-2; PGE_2; rheumatoid arthritis; t-PA

Accepted for publication 12 May 2006

Rheumatoid arthritis (RA) is characterized by the accumulation and persistence of an inflammatory infiltrate in the synovial membrane, which leads to synovitis and the destruction of the joint architecture resulting in impaired function. Periodontitis, on the other hand, may also be a threat to systemic health even though it is localized to the tooth-supporting tissues. RA and periodontitis are chronic inflammatory diseases that have similar pathological features. In both conditions, chronic inflammatory reactions occur in a boundary area composed of connective tissue and bone. Chronic inflammatory process, which occurs with complement activation, cytokine production and the release of other inflammatory cells, results with the destruction of adjacent soft and hard tissues. Although the aetiologies of these two conditions are exactly different from each other, both of them are thought to appear as a result of altered immunologic functions (Bartold et al. 2005). This similarity has led to numerous studies on the relationship between RA and periodontitis reporting controversial data (Yavuzyılmaz et al. 1992, Mercado et al. 2000, 2001).

The plasminogen activating system acts in various processes, such as tissue repair, tissue remodelling and local inflammatory reactions (Ossowski & Reich 1983). Plasminogen activators (PA) are of two types: the tissue/blood vessel-type plasminogen activator (t-PA) and the urokinase-type plasminogen activator (u-PA) (Danø et al. 1985, Saksela 1985, Åstedt 1989). t-PA and u-PA convert the proenzyme plasminogen into the broad-spectrum proteinase plasmin. Plasmin contributes directly and indirectly, via activation of latent collagenase, to the degradation and turnover of the extracellular matrix (ECM) (Kruithof 1988). The activities of the PAs in turn are regulated by plasmino-

gen activator inhibitor-1 (PAI-1) mainly produced by endothelial cells (Danø et al. 1999) and PAI-2 produced by cells such as macrophages and epithelial cells (Åstedt et al. 1986, Kruithof et al. 1995). Elevated concentrations of t-PA and PAI-2 in gingival crevicular fluid (GCF) have been reported suggesting their involvement in the aggravation of gingival inflammation (Kinnby et al. 1996). Xiao et al. (2000) have stated that t-PA and PAI-2 may play a significant role in periodontal tissue destruction and remodelling and that t-PA and PAI-2 in GCF may be used as clinical markers to evaluate the periodontal diseases and assess treatment. Significant elevations in several thrombotic predictors of cardiovascular disease (fibrinogen, t-PA antigen and fibrin D-dimer) were found in the RA patients (McEntegart et al. 2001).

Periodontitis patients exhibit elevated GCF levels of pro-inflammatory cytokines such as interleukin-1β (IL-1β), and arachidonic acid metabolites like prostaglandin E_2 (PGE_2) (Offenbacher 1996). PGE_2, the metabolite of the cyclooxygenase pathway, is the most potent mediator of alveolar bone loss in periodontitis (Offenbacher et al. 1993). PGE_2 is known to have an activity on fibroblasts and osteoclasts to induce the synthesis of matrix metalloproteinases (MMPs), IL-1β and other cytokines. PGE_2 has been detected in higher levels in gingival tissue and gingival crevicular fluid proportional to the severity of periodontal disease (Offenbacher et al. 1989). IL-1β is a central mediator of inflammation and connective tissue destruction in RA (Raymond et al. 2006). IL-1β also increases matrix degradation by inducing the production of PGE_2 in synovial cells, as well as by its role as a mediator of bone and cartilage destruction (Cutulo 2004).

It is hypothesized that periodontitis may influence RA or vice versa. Furthermore, alterations in GCF levels of some major inflammatory mediators may partly explain the possible mechanisms acting in the interactions of these two chronic inflammatory diseases. The aim of this study was, therefore, to evaluate if coexistence of periodontitis and RA has an additional effect on the severity of the two diseases and to evaluate the correlation between clinical situation and GCF levels of t-PA, PAI-2, IL-1β and PGE_2.

Material and Methods

Study population

A total number of 57 subjects were included in the present study. Twenty-three RA patients (RA group) (five males and 18 females, with a mean age of 52.65 ± 9.94 years), diagnosed according to the Criteria of the American Collage of Rheumatology (Arnett et al. 1988), were recruited from Rheumatology Clinic, School of Medicine, Ege University. The duration of RA and drugs used by these patients for the treatment of RA were recorded. All patients had been receiving prednisolone and methotrexate. Besides recording the rheumatoid factor (RF) as positive or negative, erythrocyte sedimentation rate (ESR) and circulating C-reactive protein (CRP) values were also recorded for each RA patient to determine disease activity. Exclusion criteria were any other systemic disease that can affect periodontal status, antibiotic therapy within the last 3 months and periodontal treatment within the last 6 months. Moreover, patients with less than 10 teeth were also excluded from the study. Smoking history was recorded but smokers were not excluded.

The second group of the study comprised 17 systemically healthy but periodontally diseased patients (PD group) (nine males and eight females, with a mean age of 49.12 ± 6.64 years). These patients were selected from those seeking periodontal treatment at the Department of Periodontology, School of Dentistry, Ege University. Exclusion criteria for the PD group were any known systemic disease, antibiotic treatment within the last 3 months, periodontal treatment within the last 6 months and presence of less than 10 natural teeth. In accordance with the RA group, smoking history was recorded but smokers were not excluded from the study. Clinical periodontal diagnosis of all patients in the RA and PD groups was determined and these groups were further divided into two subgroups as follows: RA-gingivitis; RA-periodontitis, healthy-gingivitis, healthy-periodontitis according to the criteria designated at the International Workshop for a Classification of Periodontal Diseases and Conditions (Mariotti 1999).

Finally, a group of volunteer individuals who were systemically and periodontally healthy were included in the study as a control group. These volunteer subjects were drawn from the staff of the Dental School and had no history of periodontal disease, i.e., probing depths < 3 mm, no attachment loss and no sign of clinical inflammation. The study protocol was explained to each subject and informed consent was received from each individual before their enrolment in the study.

GCF fluid sampling

GCF samples were obtained from buccal aspects of two inter-proximal sites in single-rooted teeth in each individual participating in the study. Inflamed sites were selected for GCF sampling. Before GCF sampling, dichotomous plaque recording was performed as present or absent and supragingival plaque was then removed carefully by sterile curettes and the surfaces were dried and isolated by cotton rolls. Filter paper strips (Periopaper, ProFlow Inc., Amytyville, NY, USA) were placed in the orifices of gingival sulcus/pocket for 30 s. Care was taken to avoid mechanical trauma and strips contaminated with blood were discarded. The absorbed GCF volume was estimated by a calibrated instrument (Periotron 8000, Oraflow Inc., Plainview, NY, USA). Then, the two strips from each patient were placed into one polypropylene tube before freezing at -40°C. The Periotron readings were converted to an actual volume (μl) by reference to the standard curve. All GCF samples were stored at -40°C until the laboratory analyses.

Clinical periodontal measurements

Subsequent to recording of dichotomous plaque index and GCF sampling, full-mouth clinical periodontal measurements were recorded at six sites per tooth (mesio-buccal, vestibule, disto-buccal, disto-lingual, lingual, mesio-lingual) including probing depth (PD), clinical attachment level (CAL) and dichotomous bleeding on probing (BOP) score as present or absent. Williams periodontal probe was used for clinical periodontal measurements and all measurements were performed by two pre-calibrated researchers (B. B. and L. K.) who were blinded to the study group of the individual.

Enzyme immunoassay

Appropriate ELISA kits were purchased for PGE_2 (Assay Designs, Ann Arbor, MI, USA), IL-1β (Biosource Interna-

tional Inc., Camarillo, CA, USA), and PAI-2 and t-PA (American Diagnostica Inc., Stamford, CT, USA). The minimum detection limits were 13.4 pg/ml, 1 pg/ml, 1 ng/ml and 1 ng/ml for PGE_2, IL-1β, PAI-2 and t-PA, respectively. GCF samples were eluted in 500 μl of PBS by shaking the tubes on an ELISA plate shaker for 45 min. For PAI-2 analyses, GCF samples were diluted 1:10, while they were used neat for t-PA, PGE_2 and IL-1β assays. The ELISA assays were carried out according to the manufacturers' recommendations and 96-well plates pre-coated with appropriate antibodies were used. Samples and standards were added to wells and incubated at room temperature for 2 h for PGE_2, IL-1β, PAI-2 and 1 h for t-PA assays. The plates were washed and detection antibody was added, and the plates were incubated for 30 min. at room temperature. After washing, conjugated antibody was added and incubated for 45 min. at room temperature. The plates were washed again and chromogen solution was added and incubated for 25 min. at room temperature. Later on, the substrate was added and incubated to develop colour change. Finally, the optical densities were read and the samples were compared with the standards. The results of PAI-2 and t-PA were expressed as nanograms for total amount calculations and as ng/μl for concentrations when adjusted for GCF volume; for PGE_2 and IL-1β total amount calculations were expressed as picograms and concentrations as pg/μl.

Laboratory markers of RA activity

Blood samples were taken from all RA patients. The erythrocyte sedimentation rate (ESR) (mm/h), C-reactive protein (CRP) (mg/l) and RF (as positive or negative) were evaluated. The ESR was determined by the Westergren method and the CRP was analysed immunologically with a commercial kit (N High sensitive CRP, DADE, Behring AG, Marburg, Germany) in accordance with the manufacturer's instructions, using a nephelometer analyser (Behring AG, Diagnostica). RF was quantitatively determined by the nephelometry technique (Dade Behring Kits and BN2 nephelometer, Marburg, Germany) and values lower than 15I U/ml were accepted as ''negative''.

Statistical analysis

ANOVA and Brown–Forsyth tests followed by post hoc Tukey's and Dunnett-C tests were used when appropriate for group comparisons of the biochemical data as well as the clinical periodontal measurements. Pearson's correlations were utilized to look at the relationships between GCF levels of the assayed inflammatory mediators and the clinical parameters of RA and periodontal disease. Pearson, χ^2 and Mann–Whitney tests were used to compare the data of RA patients exhibiting gingivitis with those exhibiting periodontitis.

Results

Clinical analyses

Clinical periodontal measurements were analysed in two steps: firstly the full-mouth recordings at six sites/tooth were compared between the study groups; secondly the clinical recordings at the GCF sampling sites were tested statistically. The mean values of clinical periodontal measurements are outlined in Table 1. The healthy control group exhibited significantly lower values in all clinical periodontal measurements ($p<0.05$). There were no significant differences between the RA and PD groups in clinical periodontal parameters ($p>0.05$).

The mean values of the laboratory markers of RA are given in Table 2. When the RA patients were subgrouped according to periodontal diagnosis, duration of RA, RF, mean values of ESR and CRP did not show significant differences between the RA gingivitis and RA periodontitis subgroups ($p>0.05$).

Biochemical analyses

The results of biochemical analysis are outlined in Table 3. Both RA and PD groups were divided into two subgroups according to periodontal diagnosis (gingivitis/periodontitis) and the biochemical data obtained in GCF samples were compared statistically between the resultant five study groups. GCF total amount of PGE_2 in the RA-periodontitis subgroup was significantly higher than that of the RA-gingivitis subgroup ($p=0.018$), while no significant difference was found in the other biochemical parameters. When the RA-gingivitis and healthy-gingivitis sub-groups were compared, no significant differences were found in the GCF values of the assayed mediators ($p>0.05$). There was no significant difference between the RA-periodontitis and healthy-periodontitis sub-groups in any of the studied parameters ($p>0.05$). The t-PA total amount and concentration values in the

Table 1. Clinical characteristics of the study groups

	Group 1: Rheumatoid arthritis	Group 2: Periodontal disease	Group 3: Healthy control
n	23	17	17
Male/female	5/18	9/8	3/14
Age (years)	52.65 ± 9.9	49.12 ± 6.6	40.65 ± 6.7*
Smoker/non-smoker	5/18	6/11	1/16
PI (%) (full mouth)	80 ± 27	93 ± 00	30.2 ± 11.5*
PD (mm) (full mouth)	2.3 ± 0.5	2.8 ± 0.8	1.3 ± 0.3*
CAL (mm) (full mouth)	2.6 ± 0.5	3.3 ± 1.2	0.4 ± 0.7*
BOP (%) (full mouth)	63 ± 29	63 ± 22	5.3 ± 2.5*
PI (%) (sample sites)	83 ± 24	90 ± 12	0*
PD (mm) (sample sites)	2.5 ± 0.7	3.0 ± 1.0	1.8 ± 0.4*
CAL (mm) (sample sites)	2.7 ± 0.9	3.3 ± 1.5	0*
BOP (%) (sample sites)	0.72 ± 0.4	0.65 ± 0.3	0*

All data presented are mean ± standard deviation values.
*Significantly lower than the other groups; $p<0.05$.
PD, probing depth; BOP, bleeding on probing; PI, plaque index; CAL, clinical attachment level.

Table 2. Laboratory markers of the RA patients

	RA patients ($n = 23$)	RA-gingivitis ($n = 13$)	RA-periodontitis ($n = 10$)
Duration of RA (years)	16.30 ± 10.4	16.69 ± 12.01	15.80 ± 8.3
ESR (mm/h)	17.60 ± 11.9	15.00 ± 6.2	21.00 ± 16.5
CRP (mg/l)	1.00 ± 1.4	0.86 ± 0.72	0.84 ± 0.4
RF (+/−)	17/6	9/4	8/2

No significant difference was found between RA-gingivitis and RA-periodontitis patients with regard to the characteristics of rheumatoid arthritis ($p > 0.05$).
RA, rheumatoid arthritis; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor.

Table 3. Biochemical data obtained in GCF samples

	RA-gingivitis ($n = 13$)	RA-periodontitis ($n = 10$)	Healthy-gingivitis ($n = 10$)	Healthy-periodontitis ($n = 7$)	Healthy control ($n = 17$)
GCF volume (μl)	0.55 ± 0.27†	0.84 ± 0.37†‡	0.43 ± 0.24	0.85 ± 0.69†	0.3 ± 0.19
t-PA total amount (ng)	0.44 ± 0.26†	0.67 ± 0.38†‡	0.31 ± 0.37†	0.39 ± 0.49†	0.01 ± 0.0
t-PA concentration (ng/μl)	1.04 ± 0.92†	0.92 ± 0.56†	0.62 ± 0.41†	0.68 ± 0.83†	0.08 ± 0.3
PAI-2 total amount (ng)	1.97 ± 1.17†	2.51 ± 1.44†	1.63 ± 0.86	1.46 ± 1.14	1.04 ± 0.7
PAI-2 concentration (ng/μl)	4.27 ± 2.98	3.46 ± 2.05	3.83 ± 1.42	3.05 ± 3.87	4.01 ± 3.5
t-PA/PAI-2	0.28 ± 0.21†	0.30 ± 0.18†	0.42 ± 0.13†	0.48 ± 0.17†	0.01 ± 0.04
IL-1β total amount (pg)	0.003 ± 0.003	0.16 ± 0.49†‡	0.003 ± 0.004	0.81 ± 2.04†	0.014 ± 0.045
IL-1β concentration (pg/μl)	0.007 ± 0.008	0.14 ± 0.40	0.005 ± 0.003	0.61 ± 1.46	0.05 ± 0.1
PGE_2 total amount (pg)	86.63 ± 22.54	113.78 ± 24.33*†‡	90.89 ± 12.70†	110.66 ± 67.4†	70.6 ± 16.3
PGE_2 concentration (pg/μl)	185.69 ± 74.61	166.43 ± 85.43	243.66 ± 85.81	233.55 ± 19.46	443.9 ± 561.6

All data presented are mean ± standard deviation values.
*Significantly higher than the RA-gingivitis group; $p < 0.05$.
†Significantly higher than the healthy control group; $p < 0.05$.
‡Significantly higher than the healthy-gingivitis group; $p < 0.05$.
RA, rheumatoid arthritis; GCF, gingival crevicular fluid; t-PA, tissue-type plasminogen activator; PAI-2, plasminogen activator inhibitor-2; IL-1β, interleukin-1β; PGE_2, prostaglandin E_2.

RA and PD groups were significantly higher than that of the healthy control group ($p < 0.05$). PAI-2 total amounts in the RA-gingivitis and RA-periodontitis subgroups were significantly higher than that of the healthy control group ($p < 0.05$). IL-1β total amount in the RA-periodontitis subgroup was higher than that of the healthy control group ($p < 0.05$). PGE_2 total amount values in the RA-periodontitis, healthy-gingivitis and healthy-periodontitis sub-groups were significantly higher than that of the healthy control group ($p < 0.05$).

In the RA group, Spearman's correlation analysis revealed no significant correlation between the laboratory markers of RA and the biochemical parameters derived from the analyses in GCF samples or the clinical periodontal recordings ($p > 0.05$) (data not shown).

Discussion

RA and chronic periodontitis are common, chronic inflammatory diseases with many pathological similarities. In both diseases, a chronic inflammatory process occurs in a confined space (joint or gingival crevice, respectively), leading to destruction of adjacent bone. Studies have revealed a remarkably parallel array of mediators, including prostaglandins, cytokines, MMPs and cell types (Bozkurt et al. 2000, Mercado et al. 2003). Both diseases are multifactorial and RA patients generally receive intensive anti-inflammatory therapy suppressing prostaglandin production, thereby limiting periodontal disease progression (Greenwald & Kirkwood 1999). In the present study, we have analysed clinical condition as well as GCF levels of some major inflammatory mediators in 23 RA patients, 17 systemically healthy patients with periodontal disease, and 17 systemically and periodontally healthy individuals. To our knowledge, this is the first study evaluating t-PA, PAI-2, PGE_2 and IL-1β levels in GCF samples of RA patients. Total amounts of cytokines in GCF sample per sampling time have been suggested as a better indicator of relative GCF constituent activity rather than concentration because concentrations are directly affected by the volume of sample (Lamster et al. 1986). Therefore, the present study is mainly based on total amount data although both total amounts and concentrations are calculated.

Studies correlating severity of RA and periodontal disease are sparse and controversial findings have been reported (Sjöström et al. 1989, Tolo & Jorkend 1990, Yavuzyılmaz et al. 1992, Kasser et al. 1997, Bozkurt et al. 2000, Mercado et al. 2000, 2001). Sjöström et al. (1989) suggested that the periodontal findings in RA patients were similar to those of the control group. In contrast, Tolo & Jorkend (1990) indicated increased alveolar bone loss in RA patients. Mercado et al. (2000) have reported that individuals with periodontitis are four times more likely to have a self-reported history of RA. In another study, clinical periodontal measurements and clinical parameters of RA have been recorded in 65 consecutive RA patients as well as in the control group from which ESR and serum levels of CRP in RA patients were suggested to be principal parameters that could be associated with alveolar bone loss (Mercado et al. 2001). Miranda et al. (2003) recorded clinical periodontal findings and ESR and CRP in 32 patients with

juvenile idiopathic arthritis (JIA) and 24 controls. They reported significantly higher values of ESR and CRP in the arthritis group and, moreover, concluded that adolescents with JIA presented more attachment loss than systemically healthy controls, in spite of similar plaque and bleeding index values. However, Reichert et al. (2006) concluded that JIA is not a risk factor for periodontitis.

In an intervention study involving 42 RA patients assigned to two groups, the possible effects of periodontal treatment were evaluated; the first group received oral hygiene instructions and professional tooth cleaning, while the second group received full-mouth scaling and root-planing (SRP) (Ribeiro et al. 2005). The authors reported significant reductions in ESR in the second group while RF was similar in both groups, and concluded that periodontal treatment with SRP might have a reducing effect on ESR.

In the present study, however, we did not find any significant differences in clinical periodontal measurements between the RA patients and the systemically healthy periodontal disease group, although a larger scale study may reveal significant differences. Moreover, when the RA patients were sub-grouped according to periodontal diagnosis, ESR, CRP or RF values did not show significant differences between the RA-gingivitis and RA-periodontitis groups. As is shown in Table 2, the mean ESR and CRP levels were normal, suggesting that the patients are all in remission period, probably because of their therapeutic regimens. RA patients were all using prednisolone and methotrexate regularly and the duration of the disease was rather long (mean duration of drug usage: 16.30 years). A long duration of RA may be expected to increase the risk of periodontal attachment loss, but on the other hand, long-term usage of these drugs may have had an inhibitory effect on periodontal tissue destruction, thereby resulting in the lack of difference in the severity of periodontal diseases between the RA group and the systemically healthy but periodontally diseased patients.

Circulating CRP level has been suggested to be correlated with the severity of periodontal disease expressed in terms of clinical periodontal measurements (Ebersole et al. 1997, Gleissner et al. 1998). Our present findings do not provide support for the hypothesis that serum levels of CRP are associated with periodontal attachment loss. In fact, serum levels of CRP may well be affected by numerous factors such as sub-clinical virus infections. Therefore, high variability of its circulating level discourages its application as a reliable marker for periodontal tissue destruction. Data originating from cross-sectional studies are particularly open for discussion, and thus, verification of persistence of the increase in CRP level is of utmost importance. Moreover, the lack of uniformity in classifying various forms of RA and periodontal disease makes it difficult to compare the data from different studies.

The plasminogen activating system is associated with fibrinolysis and is important for ECM degradation and remodelling. Plasmin acts directly on ECM by cleaving non-collagenous ECM proteins and also indirectly by activating a whole range of other enzymes, including MMPs. When our present data derived from GCF analysis are considered, all patient groups exhibited significantly elevated t-PA levels compared with the healthy control group, while RA-periodontitis patients exhibited the highest level. This increase in t-PA level may also be related with the suggested role of MMPs in the pathogenesis of RA. PAI-2 levels, on the other hand, showed significant increases only in the RA group compared with the healthy control group. However, there were no significant differences in t-PA or PAI-2 levels between the RA group and the systemically healthy periodontal disease group. Our findings support the hypothesis that the PA system is involved in deterioration of periodontal status and are in line with the previous reports (Kinnby et al. 1996, Xiao et al. 2000).

Offenbacher et al. (1993) have demonstrated that PGE_2 levels increase from health to gingivitis, and reach very high concentrations during periods of periodontal disease progression. Consistent with the literature, both of the periodontitis groups in the present study showed higher GCF levels of PGE_2 than both of the gingivitis groups and the healthy control. Prostaglandins, particularly PGE_2, are inducers of cytokine production by various cells and may be responsible for some of the bone resorption and cartilage destruction seen in RA (Dayer et al. 1986). PGE_2 acts on fibroblasts and osteoclasts to induce the synthesis of MMPs, IL-1β and other cytokines, which are crucial for tissue turnover and degradation in periodontitis. Accordingly, our data revealed the highest PGE_2 level in the RA-periodontitis group, which was significantly higher than the healthy control and also the RA-gingivitis group, but similar to the healthy-periodontitis group. The RA-periodontitis group also showed significantly higher IL-1β levels than the healthy control group. RA patients regularly use non-steroidal anti-inflammatory drugs (NSAIDs), which suppress prostaglandin synthesis and secretion, whereas methotrexate and corticosteroids have inhibitory effects on IL-1 (Emery 2006). Recently, Havemose-Poulsen et al. (2006) reported similar levels of plaque and periodontal inflammation in young adults with RA and healthy controls despite treatment with anti-inflammatory/antirheumatic medications. As our data revealed the highest PGE_2 total amount in the RA-periodontitis group, it may be speculated that if these patients had not been receiving NSAIDs and corticosteroids the PGE_2 level in these patients would have been even much higher. Havemose-Poulsen et al. (2005) documented that patients with aggressive periodontitis and types of arthritis exhibit similar components of blood cytokines distinguishing them from individuals free of disease. Clinical periodontal situations of the patients were not presented in that study, yet our present findings are in line revealing similar GCF levels of cytokines in RA and PD groups which are significantly higher than the healthy control group.

In conclusion, our present data provide further evidence for the hypothesis that a disregulation of molecular pathways in the inflammatory response underlies both RA and periodontitis. Considering the similarity in GCF levels of PGE_2 and IL-1β in RA and PD groups despite the long-term usage of corticosteroids and NSAIDs, our findings suggest that RA patients may have a propensity to overproduce these inflammatory mediators. These immunosuppressive and NSAIDs suppress the production of inflammatory mediators in RA patients. Therefore, it may be speculated that without long-term NSAID suppression RA patients would exhibit higher GCF amounts of these mediators that could possibly lead to increased periodontal destruction. However, the coexistence of RA and periodontitis

does not seem to have a significant influence on the clinical periodontal findings or systemic markers of RA. Further longitudinal clinical studies are required to better clarify the nature and possible mechanisms of interactions between these two chronic inflammatory diseases.

Acknowledgements

The authors greatly appreciate the contribution of Mehmet Durmuşcan in performing the ELISA assays.

References

Arnett, F. C., Edworthy, S. M., Bloch, D. A., McShane, D. J., Fries, J. F., Cooper, N. S., Healey, L. A., Kaplan, S. R., Liang, M. H. & Luthra, H. S. (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis and Rheumatism* **31**, 315–324.

Åstedt, B. (1989) No cross reaction between circulating plasminogen activator and urokinase. *Thrombosis Research* **14**, 535–539.

Åstedt, B., Hagerstrand, I. & Lecander, I. (1986) Cellular localization in placenta of placental type plasminogen activator inhibitor. *Thrombosis and Haemostasis* **56**, 63–65.

Bartold, P. M., Marshall, R. I. & Haynes, D. R. (2005) Periodontitis and rheumatoid arthritis: a review. *Journal of Periodontology* **76**, 2066–2074.

Bozkurt, F. Y., Berker, E., Akkuş, S. & Bulut, Ş. (2000) Relationship between interleukin-6 levels in gingival crevicular fluid and periodontal status in patients with rheumatoid arthritis and adult periodontitis. *Journal of Periodontology* **71**, 1756–1760.

Cutulo, M. (2004) IL-1Ra: its role in rheumatoid arthritis. *Rheumatismo* **56**, 41–45.

Danø, K., Andreasen, P., Grondahl-Hansen, J., Kristensen, P., Nielsen, L. S. & Skriver, L. (1985) Plasminogen activators, tissue degradation and cancer. *Advances in Cancer Research* **44**, 139–266.

Danø, K., Romer, J., Nielsen, B. S., Pyke, C., Rygaard, J. & Lund, L. (1999) Cancer invasion and tissue remodeling – cooperation of protease systems and cell types. Review article. *APMIS* **107**, 120–127.

Dayer, J., de Rochemonteix, B., Burrus, D., Demczuk, S. & Dinarello, C. A. (1986) Human recombinant IL-1 stimulates collagenase and prostglandin production by human synovial cells. *Journal of Clinical Investigation* **77**, 645–648.

Ebersole, J., Machen, R., Steffen, M. J. & Willmann, D. E. (1997) Systemic acute phase reactants, C-reactive proteins and haptoglobulin in adult periodontitis. *Clinical and Experimental Immunology* **107**, 347–352.

Emery, P. (2006) Treatment of rheumatoid arthritis. *British Medical Journal* **21**, 152–155.

Gleissner, C., Willershausen, B., Kaesser, U. & Bolten, W. W. (1998) The role of risk factors for periodontal disease in patients with rheumatoid arthritis. *European Journal of Medical Research* **3**, 387–392.

Greenwald, R. A. & Kirkwood, K. (1999) Adult peridoontitis as a model for rheumatoid arthritis with emphasis on treatment strategies. *Journal of Rheumatology* **26**, 1650–1653.

Havemose-Poulsen, A., Sorenson, L. K., Stoltze, K., Bendtzen, K. & Holmstrup, P. (2005) Cytokine profiles in peripheral blood and whole blood cell cultures associated with aggressive periodontitis, juvenile idiopathic arthritis, and rheumatoid arthritis. *Journal of Periodontology* **76**, 2276–2285.

Havemose-Poulsen, A., Westergaard, J., Stoltze, K., Skjodt, H., Danneskiold-Samsoe, B., Locht, H., Bendtzen, K. & Holmstrup, P. (2006) Periodontal and hematological characteristics associated with aggressive periodontitis, juvenile idiopathic arthritis, and rheumatoid arthritis. *Journal of Periodontology* **77**, 280–288.

Kasser, U. R., Gleissner, C., Dehne, F., Michel, A., Willershausen-Zonnchen, B. & Bolten, W.W. (1997) Risk for periodontal disease in patients with longstanding rheumatoid arthritis. *Arthritis and Rheumatism* **40**, 2248–2251.

Kinnby, B., Matsson, L. & Åstedt, B. (1996) Aggravation of gingival inflammatory symptoms during pregnancy associated with the concentration of plasminogen activator inhibitor type 2 (PAI-2) in gingival fluid. *Journal of Periodontal Research* **31**, 271–277.

Kruithof, E. K. O. (1988) Plasminogen activator inhibitors – a review. *Enzyme* **40**, 113–121.

Kruithof, E. K. O., Baker, M. S. & Bunn, C. L. (1995) Biological and clinical aspects of plasminogen activator inhibitor type 2. *Blood* **86**, 4007–4024.

Lamster, I. B., Oshrain, R. L. & Gordon, J. M. (1986) Enzyme activity in human gingival crevicular fluid: considerations in data reporting based on analysis of individual crevicular sites. *Journal of Clinical Periodontology* **13**, 799–804.

Mariotti, A. (1999) Dental plaque-induced gingival diseases. *Annals of Periodontology* **4**, 7–17.

McEntegart, A., Capell, H. A., Creran, D., Rumley, A., Woodward, M. & Lowe, G. D. (2001) Cardiovascular risk factors, including thrombotic variables, in a population with rheumatoid arthritis. *Rheumatology* **40**, 640–644.

Mercado, F.B, Marshall, R. I. & Brtold, P. M. (2003) Inter-relationships between rheumatoid arthritis and periodontal disease. A review. *Journal of Clinical Periodontology* **30**, 761–772.

Mercado, F., Marshall, R. I., Klestov, A. C. & Bartold, P. M. (2000) Is there a relationship between rheumatoid arthritis and periodontal disease? *Journal of Clinical Periodontology* **27**, 267–272.

Mercado, F. B., Marshall, R. I., Klestov, A. C. & Bartold, P. M. (2001) Relationship between rheumatoid arthritis and periodontitis. *Journal of Periodontology* **72**, 779–787.

Miranda, L. A., Fischer, R. G., Sztajnbok, F. R., Figueredo, C. M. S. & Gustafsson, A. (2003) Periodontal conditions in patients with juvenile idiopathic arthritis. *Journal of Clinical Periodontology* **30**, 969–974.

Offenbacher, S. (1996) Periodontal diseases: pathogenesis. *Annals of Periodontology* **1**, 879–925.

Offenbacher, S., Heasman, P. A. & Collins, J. G. (1993) Modulation of host PGE_2 secretion as a determinant of periodontal disease expression. *Journal of Periodontology* **64**, 432–444.

Offenbacher, S., Odle, B. M., Braswell, L. D., Johnson, H.G., Hall, C.M., McClure, H., Orkin, J.L., Strobert, E.A. & Green, M.D. (1989) Changes in cyclooxygenase metabolites in experimental periodontitis in Macaca mulatta. *Journal of Periodontal Research* **24**, 63–74.

Ossowski, L. & Reich, E. (1983) Antibodies to plasminogen activator inhibit human tumor metastasis. *Cell* **35**, 611–619.

Raymond, L., Eck, S., Mollmark, J., Hays, E., Tomek, I., Kantor, S., Elliott, S. & Vincenti, M. (2006) Interleukin-1 beta induction of matrix metalloproteinase-1 transcription in chondrocytes requires ERK-dependent activation of CCAAT enhancer-binding protein-beta. *Journal of Cell Physiology* **35**, 335–340.

Reichert, S., Machulla, H. K. G., Fuchs, C., John, V., Schaller, H.-G. & Stein, J. (2006) Is there a relationship between juvenile idiopathic arthritis and periodontitis? *Journal of Clinical Periodontology* **33**, 317–323.

Ribeiro, J., Leao, A. & Novaes, A. B. (2005) Periodontal infection as a possible severity factor for rheumatoid arthritis. *Journal of Clinical Periodontology* **32**, 412–416.

Saksela, O. (1985) Plasminogen activation and regulation of pericellular proteolysis. *Biochimica Biophysica Acta* **823**, 35–65.

Sjöström, L., Laurell, L., Hugoson, A. & Hakansson, J. P. (1989) Periodontal conditions in adults with rheumatoid arthritis. *Community Dentistry and Oral Epidemiology* **17**, 234–236.

Tolo, K. & Jorkend, L. (1990) Serum antibodies and loss of periodontal bone in patients with rheumatoid arthritis. *Journal of Clinical Periodontology* **17**, 288–291.

Xiao, Y., Bunn, C. L. & Bartold, P. M. (2000) Detection of tissue plasminogen activator (t-PA) and plasminogen activator inhibitor 2 (PAI-2) in gingival crevicular fluid from healthy, gingivitis and periodontitis patients. *Journal of Clinical Periodontology* **27**, 149–156.

Yavuzyılmaz, E., Yamalık, N., Çalgüner, M., Ersoy, F., Baykara, M. & Yeniay, İ. (1992) Clinical and immunological characteristics of patients with rheumatoid arthritis and periodontal disease. *Journal of Nihon University School of Dentistry* **34**, 89–95.

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Clinical Relevance

Scientific rationale for study: Coexistence of RA and periodontitis has been suggested to increase severity of both diseases. Gingival crevicular fluid levels of major inflammatory mediators may provide an explanation for this inter-relationship.

Principal findings: Clinical periodontal findings and gingival crevicular fluid profiles of evaluated inflammatory mediators were similar in RA patients and systemically healthy periodontal disease patients despite the long-term usage of anti-inflammatory drugs in RA.

Practical implications: Coexistence of RA and periodontitis appears not to significantly affect the severity of each other, but RA patients may have a propensity to overproduce these mediators.